# Isolation and purification of hydroxyanthraquinones using the Agilent 1100 Series purification system

Application

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**Abstract**

Semi-preparative and preparative HPLC is probably the most common technique for isolation and purification of single compounds from mixtures. For example, it is applied to isolate synthesis products, to purify combinatorial chemistry library compounds or to separate isomers or enantiomers. In this Application Note we describe how another typical task for preparative HPLC, the isolation of compounds from crude natural product extracts, can be easily performed using the Agilent 1100 Series purification system.

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## Introduction

Today, isolation and characterization of single compounds from complex natural product extracts is a standard procedure for a natural product chemist. Liquid chromatography, usually in semi-preparative or preparative scale with flow rates over 10 ml/min, is the tool of choice to separate the compounds [1,2,3]. The goal is to get the desired compounds in high purity and sufficient amount for structure elucidation usually done by NMR, MS and IR spectroscopy. With the Agilent 1100 Series purification system Agilent provides a toolset for sample handling and fraction collection at flow rates up to 100 ml/min. The software allows complete control of the Agilent 220 micro plate sampler and provides an easy-to-use graphical user interface for study set-up and sample/fraction tracking. The system is complemented with the Agilent 1100 Series diode array detector for detection and spectral information and the Agilent 1100 Series MSD for mass information needed for structure elucidation. In this Application Note we describe the isolation of four hydroxyanthraquinones from the extract of rhubarb root (*Rheum palmatum*) to demonstrate how a natural product chemist would isolate and purify unknown compounds from a crude natural product extract.

## Equipment

The system included two Agilent 1100 Series preparative pumps, an Agilent 1100 Series diode array detector, an Agilent 1100 Series column organizer and an Agilent 220 micro plate sampler modified for higher flow rates. The system was controlled using the Agilent ChemStation (revision A.08.04) and the micro plate sampling software (revision A.03.02).

## Results and Discussion

### Extraction

37.6 g rhubarb root (*Rheum palmatum*) were extracted ultrasonically five times with 100 ml methanol each. The combined extracts were evaporated to give 25 ml crude extract.

### Volume overloading on the analytical column

For method development the crude extract was first applied to an analytical system. The method was optimized regarding selectivity of the compounds and run time. The chromatogram measured with the optimized method is shown in figure 1.

The concentration of the crude extract was not variable because of the extraction process . Therefore, concentration overloading was not possible and volume overloading had to be done. The results for volume overloading on the analytical column are shown in figure 2.

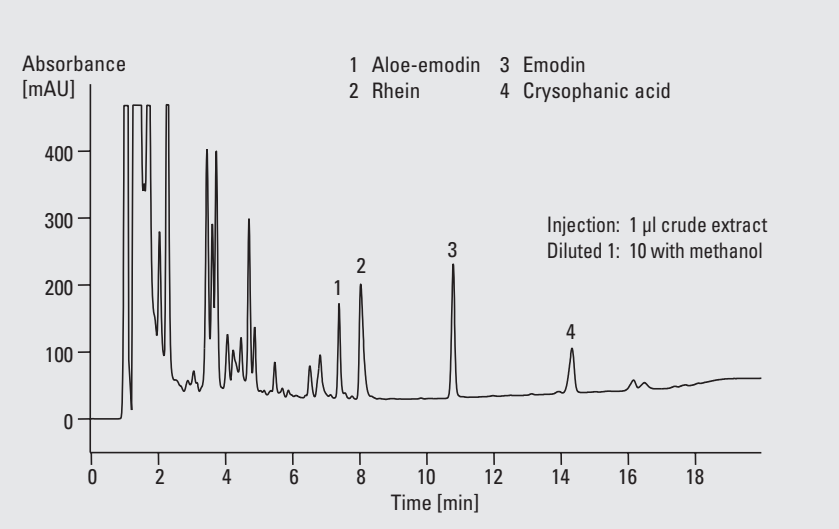


**Figure 1**
**Analytical chromatogram of crude rhubarb extract**

| | |
|---|---|
| Mobile Phases: | 0.1 % HOAc in water = A |
| | 0.1 % HOAc in ACN = B |
| Gradient: | 30 % B to 50 % B in 5 min |
| | 50 % B to 70 % B in 10 min |
| | 70 % B to 80 % B in 1 min |
| | 80 % B for 3 min |
| | 80 % B to 30 % B in 1 min |
| Stop time: | 20 min |
| Post time: | 5 min |
| Column: | Zorbax SB-C18 |
| | 3 × 150 mm, 5 µm |
| Flow: | 0.6 ml/min |
| Injection: | 5 µl |
| Column temp.: | ambient |
| UV detector: | DAD 222 nm/16 |
| | (reference off) |
| | Standard cell |
| | (10 mm pathlength) |

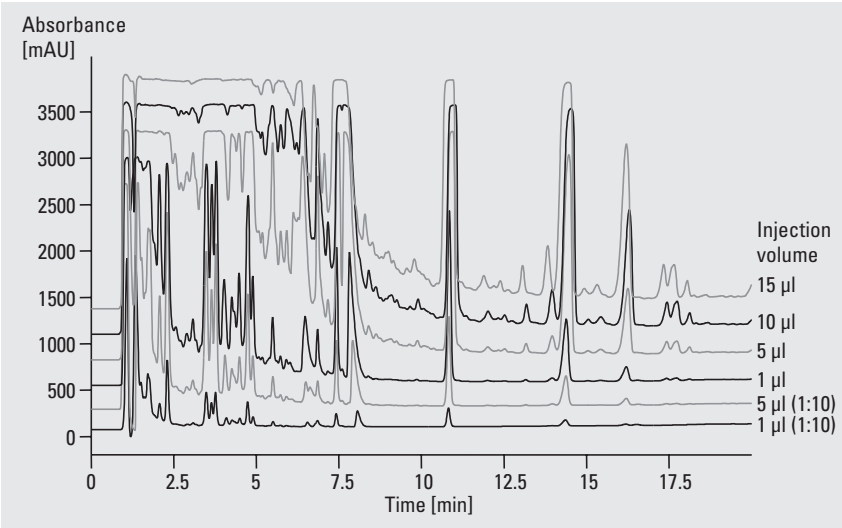


**Figure 2**
**Volume overloading on the analytical column**

### Scale up to preparative scale

The scale-up from the analytical to the preparative column was calculated using the formulae shown in figure 3.

After the first run on the preparative column the flow rate was adjusted to 25 ml/min to get similar retention times. After the adjustment was done the next preparative run was fractionated using time slices. The first 30 fractions were taken starting at 6 minutes with a slice width of 0.2 minutes. Another 20 fractions were taken starting at 14 minutes with the same slice width. The result of the preparative run and the fractionation information are shown in figure 4.

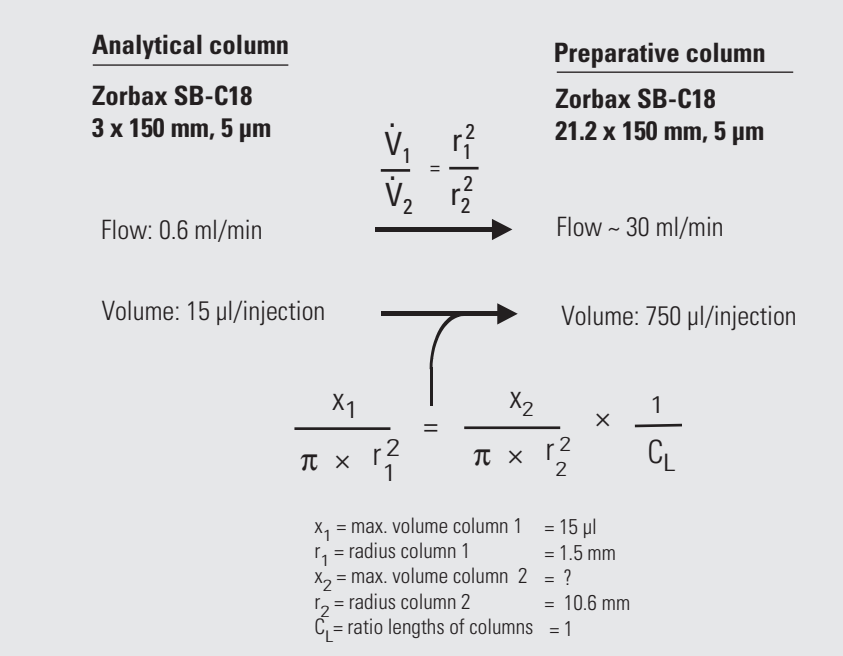


**Figure 3**
**Scale up from analytical to preparative column**

| | |
|---|---|
| Mobile Phases: | 0.1 % HOAc in water = A |
| | 0.1 % HOAc in ACN = B |
| Gradient: | 30 % B to 50 % B in 5 min |
| | 50 % B to 70 % B in 10 min |
| | 70 % B to 80 % B in 1 min |
| | 80 % B for 3 min |
| | 80 % B to 30 % B in 1 min |
| Stop time: | 20 min |
| Post time: | 5 min |
| Column: | Zorbax SB-C18 |
| | 21.2 × 150 mm, 5 µm |
| Flow: | 25 ml/min |
| Injection: | 750 µl |
| Column temp.: | ambient |
| UV detector: | DAD 222 nm/16 |
| | (reference off) |
| | Standard cell |
| | (3 mm pathlength) |

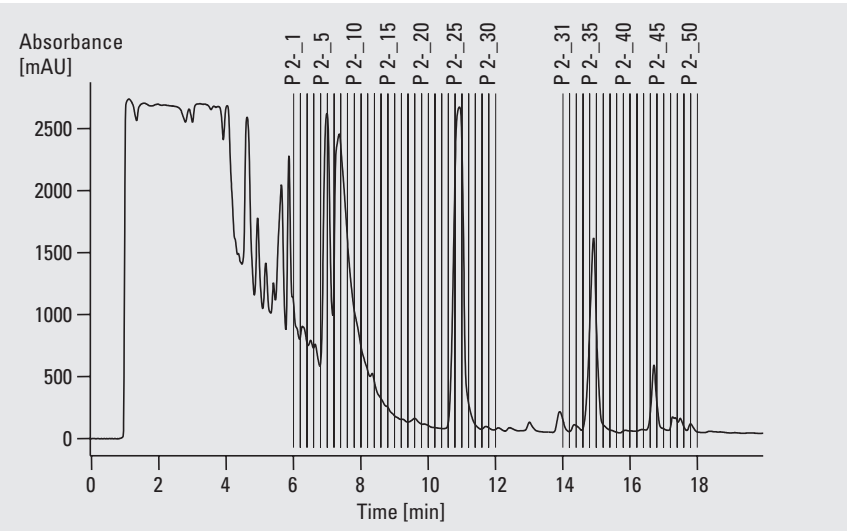


**Figure 4**
**Preparative run with fraction collection**

### Reanalysis of fractions and fraction pooling

The 50 fractions were reanalyzed using the analytical method and column to determine the purity of the compounds. The results are shown in figure 5. For the compounds aloe-emodin and rhein the recovery is low due to the poor separation of the compounds. Therefore, fractions containing both compounds were discarded to get the anthraquinones in sufficient purity. At least rhein, emodin and crysophanic acid could be isolated in sufficient purity for further NMR and MS spectrometric analysis. For aloe-emodin probably a second purification step would be necessary.

For detailed activity tests the isolated amount would not be sufficient. Therefore, 75-µl volumes of sample were injected repetitively ten times and the fractions were pooled. This could be done conveniently using the dedicated micro plate sampling software. As a result at least 20 mg of each compound were isolated in good purity, which would be enough for complete structure elucidation and primary activity tests.

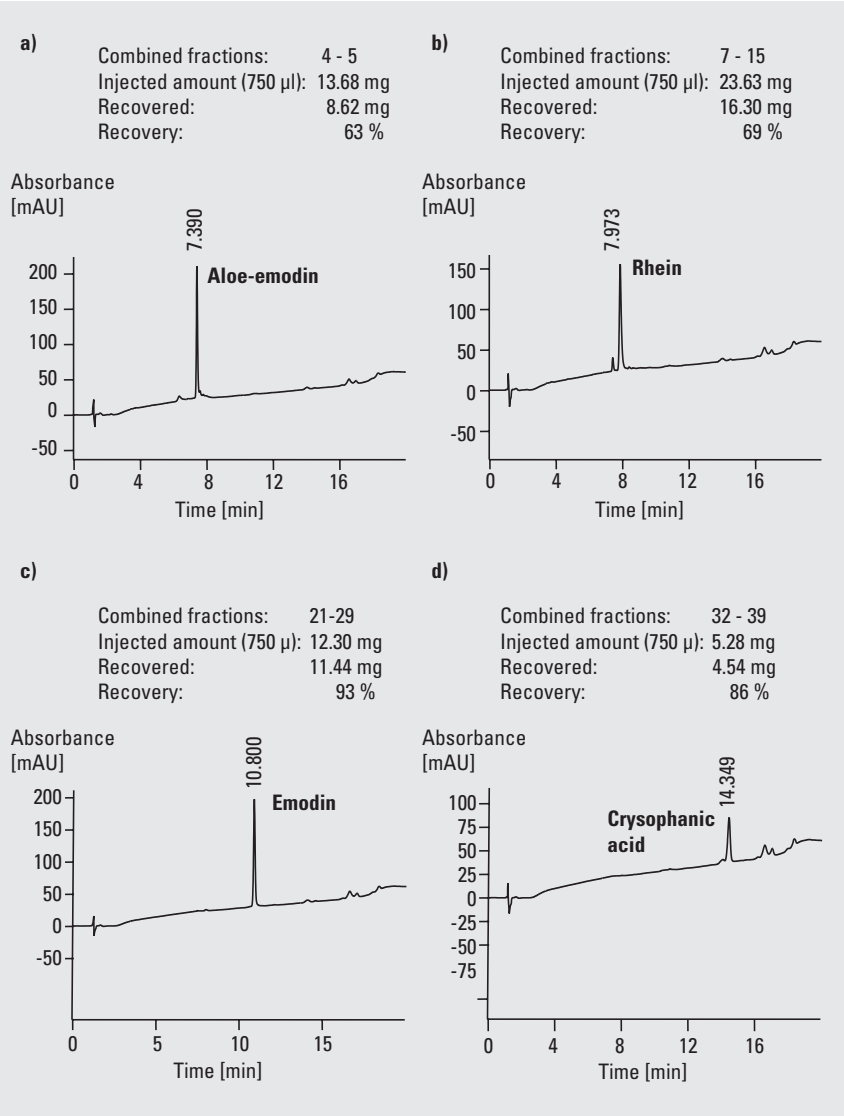


**Figure 5**
**Combined fractions of a) aloe-emodin b) rhein c) emodin d) crysophanic acid**

## Conclusion

In this Application Note we demonstrated some features of the Agilent 1100 Series purification system to isolate and purify compounds from crude natural product extracts in preparative scale. Four hydroxyathraquinones were isolated from the extract of the rhubarb root (*Rheum palmatum*) using fraction collection based on time windows. To obtain sufficient amounts of the compounds the purification was done repetitively and the collected fractions were pooled automatically. As a result, at least three of the four compounds were isolated in sufficient amount and purity for NMR and MS spectroscopy for identification and structure elucidation and for initial activity testing.

## References


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